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Adapting OECD Aquatic Toxicity Tests for Use with Manufactured Nanomaterials: Key Issues and Consensus Recommendations

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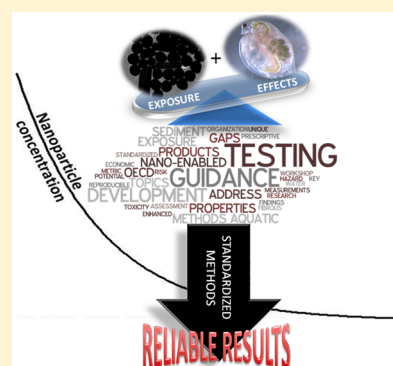
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Supporting Information

ABSTRACT: The unique or enhanced properties of manufactured nanomaterials (MNs) suggest that their use in nanoenabled products will continue to increase. This will result in increased potential for human and environmental exposure to MNs during manufacturing, use, and disposal of nanoenabled products. Scientifically based risk assessment for MNs necessitates the development of reproducible, standardized hazard testing methods such as those provided by the Organisation of Economic Cooperation and Development (OECD). Currently, there is no comprehensive guidance on how best to address testing issues specific to MN particulate, fibrous, or colloidal properties. This paper summarizes the findings from an expert workshop convened to develop a guidance document that addresses the difficulties encountered when testing MNs using OECD aquatic and sediment test guidelines. Critical components were identified by workshop participants that require specific guidance for MN testing: preparation of dispersions, dose metrics, the importance and challenges associated with maintaining and monitoring exposure levels, and the need for reliable methods to quantify MNs in complex media. To facilitate a scientific advance in the consistency of nanoeotoxicology test results, we identify and discuss critical considerations where expert consensus recommendations were and were not achieved and provide specific research recommendations to resolve issues for which consensus was not reached. This process will enable the development of prescriptive testing guidance for MNs. Critically, we highlight the need to quantify and properly interpret and express exposure during the bioassays used to determine hazard values.



INTRODUCTION

The rapidly accelerating development and implementation of nanotechnology has inspired vigorous debate about the adequacy of current regulatory frameworks for assuring the safe deployment of manufactured nanomaterials (MNs) in the

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commercial marketplace.^{1–4} A critical aspect of these debates is whether standard test protocols currently used in risk assessment are fully adequate for testing the hazard potential of MNs.^{5,6} Standardized testing protocols, and the guidance documents that describe them, are a critical component of risk assessment and regulatory processes that enable placement of chemical substances on the market. These test protocols describe specific techniques and methods for the collection and analyses of data with the goal of quantitatively describing, under controlled laboratory conditions, the release, fate, transport, transformation, exposure, and toxicity of chemical substances. The Organisation for Economic Cooperation and Development (OECD) has promulgated internationally accepted test guidelines (TGs) that are used for these purposes. A subset of these TGs focus on toxicity in aquatic, sediment, and soil organisms and constitute the OECD's Test Guidelines Section 2: "Effects on Biotic Systems".^{7–10}

Several recent publications focused on aquatic and sediment ecotoxicity assay methods commonly used in regulatory testing suggest that these methods are generally adequate for testing of MNs but discuss the need for additional guidance to improve their applicability for hazard assessment of MNs.^{8–14} The critical issue is that aquatic ecotoxicity testing with MNs involves exposure of test organisms to colloids or particle–sediment mixtures rather than solely to dissolved chemicals, for which the OECD TGs were originally intended. MNs in test media typically undergo extensive agglomeration, settling, particle dissolution, and transformations during exposure and medium-renewal periods.^{9,15} These transformation processes depend in part on the intrinsic properties of the MN, the concentration of the MN, and the composition of the medium. The resulting variability in exposure presents unique challenges for exposure–response estimation. Alternate dose metrics based on particle number, surface area, or body burden in addition to mass concentration might be informative; however, metrics other than mass concentration are not generally considered within current risk assessment frameworks. Dissolution and ion release from MNs during testing, as often observed for silver and zinc oxide MNs,^{16,17} further complicate dosimetry because the resulting exposures potentially involve both MNs and dissolved species. Concentration-dependent MN agglomeration, settling, and dissolution also present significant measurement and monitoring challenges, both logistically and methodologically. These MN behaviors often alter exposure levels beyond $\pm 20\%$ of the initial (measured) or nominal concentration during an aquatic bioassay, a specification in many TGs hereafter termed the "20% exposure specification". While MNs released from nanoenabled products may differ substantially from their as-produced form (e.g., CNTs released to the environment from polymer nanocomposites may be partly or fully encased in component polymers^{18–21}), the focus in this review is on as-produced MNs.

Herein we discuss the findings of a workshop focused on drafting an OECD guidance document (GD) on Aquatic (and Sediment) Toxicology Testing of Nanomaterials, which provides necessary amendments to existing OECD aquatic toxicity test methods and is an OECD project approved in 2013. This meeting, held at the U.S. Environmental Protection Agency (EPA) in Washington, DC, in July 2014, was attended by 23 experts from seven countries. We discuss in depth the key limitations of current aquatic bioassay study designs for testing of MNs and knowledge gaps that preclude or hinder the

development of prescriptive, broadly applicable aquatic toxicity standard tests for MNs, and we suggest research to address these issues. Each of the following topics raised at the meeting is critically discussed: key considerations for testing the aquatic toxicity of MNs; the feasibility of conducting tests with MNs that meet the 20% exposure specification; dosimetry and interpretation concerns for MNs; and challenges with testing of MNs in sediments. We highlight issues where consensus was and was not reached during the workshop and subsequent discussions with workshop participants and recommend research to resolve topics where consensus was not reached. The discussions and viewpoints expressed by the workshop participants are summarized and inform but are not binding toward the development of the OECD GD described above. The workgroup participants agreed to define MNs broadly as solid-phase substances having one dimension between 1 and 100 nm. While there are more detailed definitions (e.g., the European Commission-proposed definition²²), our intent is to avoid limiting the workgroup findings to current MN definitions that may change. The more specific terminology used here (e.g., particle size, dissolution, agglomeration, aggregation, etc.) generally follow OECD documents on MNs.²³

■ KEY CONSIDERATIONS RELATED TO AQUATIC NM TOXICITY TESTING

The Importance of Standard Terminology. Workshop participants strongly agreed on the importance of using precise terminology when describing results from nanoecotoxicity tests. The absence of terminology in ecotoxicology TGs specific to (nano) particles, colloids, dispersions, and suspensions further complicates the conduct of standard aquatic ecotoxicity tests with MNs.²⁴ For example, MN suspensions have been erroneously called dissolved MNs rather than dispersed or suspended MNs. The operational definition of "dissolved" substances varies significantly among different fields, and there are environmental and mechanistic definitions that are partially related to the operational definitions;²⁵ a more detailed discussion of this topic is available in the [Supporting Information](#). It is thus critical to make a distinction among the terms "suspension" and "dispersion" versus "solution." As the term "solution" suggests that the MNs are dissolved in the aqueous test medium, the terms "suspension" and "dispersion" are favored. This is especially important because "true" dissolution of MNs into their component ions is an important process in environmental fate and ecotoxicology. For instance, some dispersed or suspended MNs will subsequently fully or partly dissolve to their constituent ions over the exposure time of nanoecotoxicity tests, and this must be taken into account in interpreting data. Consistent use of terminology can therefore minimize misinterpretation of reported results.

For the past two decades, guidance for aquatic toxicity testing for hazard assessment has included a distinction in the terminology used to describe adverse effects. Intrinsic toxicity is derived from exposure to dissolved molecules and is distinct from adverse physical effects.²⁶ Physical effects can be manifested as attachment of insoluble material to the exterior of an organism as micelles, aggregated particles, or a flocculent and lead to adverse effects from fouled respiratory surfaces, impaired mobility, and feeding (daphnids) or light attenuation (algae). Intrinsic toxicity is the focus of aquatic hazard assessment based on the concept that the dissolved molecule represents the most relevant exposure condition for aquatic

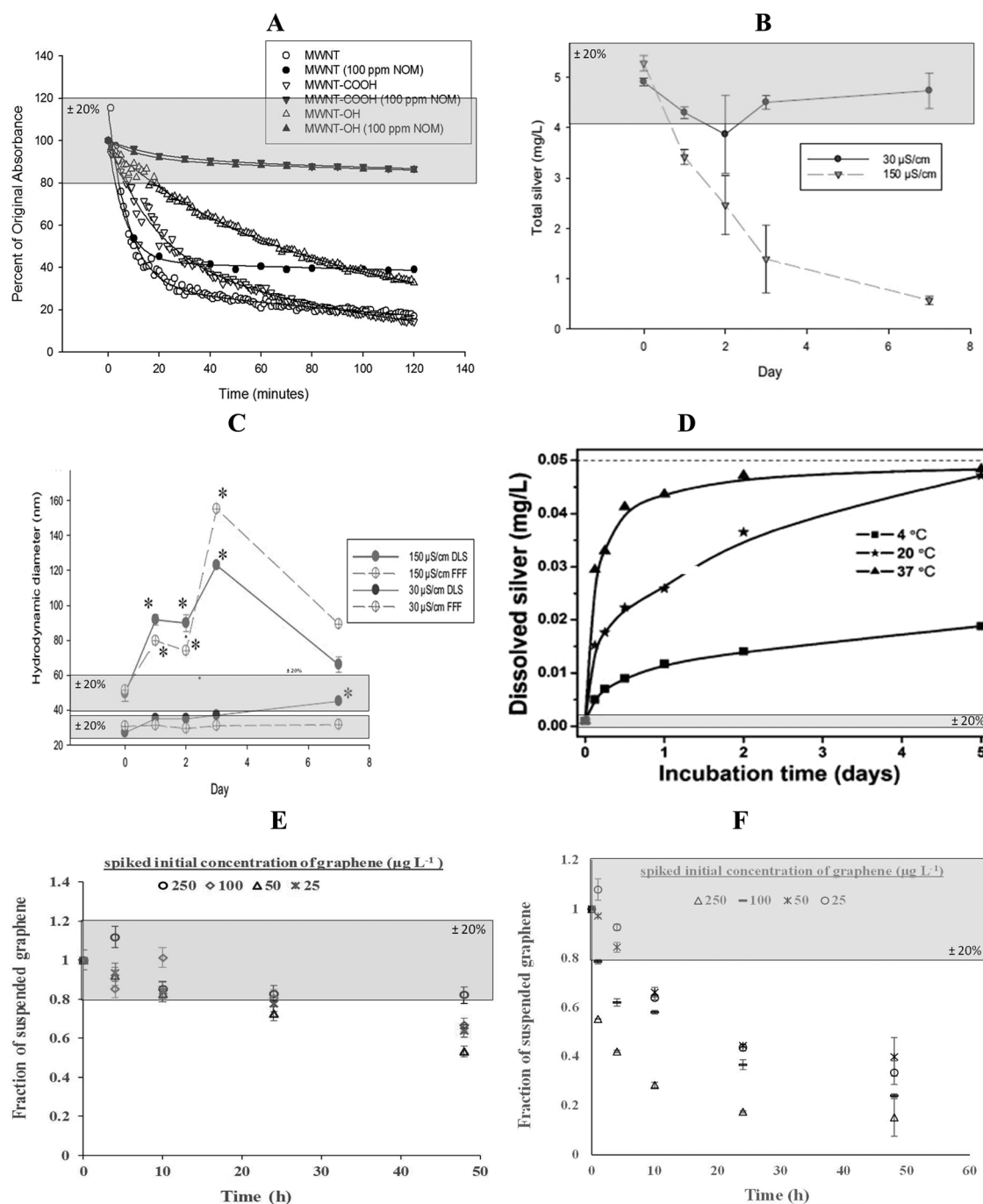


Figure 1. Examples of changes in nanoparticle stability (transformations) in environmentally relevant test media, with gray regions representing $\pm 20\%$ of the original value. (A) Different settling rates and stable concentrations of carbon nanotubes with different surface modifications and natural organic matter (NOM). A concentration of 100 ppm indicates 100 mg/L. (B, C) Impact of greater ionic strength in the medium on (B) the nanosilver concentration and (C) the hydrodynamic diameter. (D) Increase in the dissolved concentration of nanosilver with time at different temperatures. (E, F) Impact of test organisms on nanoparticle stability: while graphene settling is relatively low in absence of test organisms (E), the presence of *Daphnia magna* increases settling (F). Error bars in (C), (E), and (F) represent standard deviations of triplicate measurements, while the data points indicate the mean values. Panel (A) was reprinted with permission from ref 61. Copyright 2008 SETAC. Panels (B) and (C) were reprinted from ref 166. Panel (D) was reprinted from ref 16. Copyright 2010 American Chemical Society. Panels (E) and (F) were reprinted from ref 53. Copyright 2013 American Chemical Society.

toxicity testing and undissolved material is excluded from tests to avoid physical effects.^{27,28} Since aquatic exposures to MNs may include both dissolved and solid phases, additional effort is required to distinguish “intrinsic” toxicity from physical effects. In tests with MNs, particulate uptake has the potential to exert toxic effects that are not solely physical. Carefully designed

control experiments are essential for making a distinction and avoiding misinterpretations²⁹ and need to be incorporated into future work, including evaluations of how and when to include the hazard from physical effects into aquatic risk assessment.

In addition, the use of terms related to an “equilibrium” being reached among multiple phases including organism

tissues (i.e., bioconcentration factor, bioaccumulation factor, biota–sediment accumulation factor, etc.) is discouraged⁹ or, at a minimum, needs to be better qualified. The use of these terms may result in an inaccurate comparison between organism accumulation of MNs and hydrophobic organic contaminants (HOCs) or dissolved metals. Bioaccumulation of HOCs is related to passage through biological membranes via passive diffusion or active uptake through ion channels or carrier-mediated transport.³⁰ For MNs, however, results show that absorption into organism tissues is typically limited. For example, ingestion of carbon-based MNs by aquatic organisms often leads to high ingested concentrations present only in the gut tract with nondetectable absorption into systemic circulation,^{18,31,32} while many HOCs are concentrated in the lipid fraction of organisms.^{33–36} In addition, changes in the octanol–water partition coefficients were not shown to correlate with changes in accumulation of multiwall carbon nanotubes (MWCNTs) by a benthic organism (*Lumbriculus variegatus*) or an earthworm (*Eisenia fetida*).³⁷ An OECD document on sample preparation and dosimetry indicated that the OECD TG for octanol–water partition coefficients is unlikely to be directly applicable for use with MNs,²³ a conclusion also reached by others.³⁸

MN Behavior in Test Systems. The behaviors of MNs in aqueous media impact the accuracy and reproducibility of results derived from OECD ecotoxicity methods in that they are more dynamic and not predictable by traditional methods of partitioning and bioavailability. MNs are similar in concept to solid particulate chemicals or mixtures described as “difficult substances”.²⁷ For example, MNs may agglomerate, settle from suspension, and/or dissolve^{18,39} (Figure 1). Moreover, these behaviors are greatly influenced by the test medium and other factors such as the MN number concentration. Media with higher ionic strength, and especially higher concentrations of divalent and trivalent metal ions, result in higher rates of agglomeration and settling of MNs from suspension, with stabilization mechanisms playing a role.⁴⁰ Silver nanoparticles (AgNPs) provide an example of an MN that undergoes transformations in aqueous media; AgNPs may form silver chloride or silver sulfide particles if the medium contains chloride or sulfur, respectively, and these modified particles can be significantly less toxic than unmodified AgNPs.^{15,41,42} Silver nanoparticles also interact with natural organic material (NOM), oxidize, and dissolve,^{15,29} which influences their surface chemistry, dissolution, aggregation, and toxicity.^{43–46} Formation of AgNPs from reduction of ions can also occur in aquatic media.^{47,48} Agglomeration and settling cause increased heterogeneity in the test vessel, with higher mass concentrations toward the bottom of the container. The procedure used to disperse MNs in the aqueous medium and the MN concentration dispersed can also impact the general dispersion stability and heterogeneity in the test container as well as the rate of agglomeration.⁴⁹ Thus, the assay results for MNs are often more sensitive to the dispersion and mixing steps than those for dissolved metals or HOCs. Additionally, washing procedures to purify MNs can influence their chemistry and behavior when the coating is weakly bound to the MN surface.⁵⁰ All of these changes to the MN distribution could lead to inaccurate or inconsistent organism exposure.²⁹

Monitoring and Quantifying MN Exposure. The current lack of widely available routine measurement methods with known accuracy, precision, and method performance requirements for quantifying the mass concentration and dispersion

state of MNs in test media further complicates MN testing. While quantitative measurements of the distribution of MNs in the test containers throughout bioassays are critical for understanding variable test results, such measurements are rarely performed (exceptions include refs 51–54). When nonstandardized methods are used, they are often experimental in nature and not easily implemented by testing laboratories. Describing quantification methods for each type of MN is beyond the scope of this paper but has been considered elsewhere.^{55–58} Quantifying the MN concentration in the test suspension is most difficult for lower MN concentrations (i.e., $\mu\text{g L}^{-1}$) with most methods; while a promising recent study used atomic force microscopy to produce concentrations down to micrograms per liter,⁵⁹ this process has not yet been standardized and is not available to most ecotoxicology laboratories for routine analysis. It is possible to measure aqueous-phase concentrations of carbon nanomaterials (CNMs) greater than 1 mg L^{-1} using techniques such as UV/vis absorption spectroscopy^{60,61} and gravimetric analysis.^{31,62,63} While some methods for quantifying lower CNM concentrations are described in the literature, these methods detect only specific types of carbon nanotubes (CNTs)⁶⁴ or additional work is needed to standardize the methods.^{65–67} Metal and metal oxide MNs can be quantified in bulk by elemental analysis (e.g., by inductively coupled plasma mass spectrometry (ICP-MS)) at low concentrations. Separation methods such as ultrafiltration, centrifugation, and dialysis membrane techniques can be used to distinguish between unagglomerated, agglomerated, and dissolved MNs but have not yet been standardized.^{16,29,68,69} The applicability and reproducibility of these separation methods will be assessed by an OECD group developing a test guideline for measuring MN dissolution. Emerging techniques such as single-particle ICP-MS^{70–75} and liquid nebulization/differential mobility analysis⁷⁶ can distinguish among some of these different transformations for metal-containing MNs. However, they require standardization and have MN-dependent limitations because their lowest measurable MN sizes are above 1 nm, and thus, their practical application for routine hazard testing has not yet been demonstrated. Recently, Mader et al.⁷⁶ addressed this issue by providing a framework for evaluating the performance of new MN measurement methods.

The Role of Standardized Hazard Testing in MN Risk Assessment. The different behaviors of MNs in comparison with soluble chemicals such as HOCs and dissolved metals have raised questions about the common practice of separately assessing hazard and exposure. While significant progress has been made toward understanding the environmental fate and transformation of MNs^{15,77–80} and obtaining the basic information required to estimate exposure,⁸¹ work is still ongoing to develop models to predict the fate and hazard of MNs on the basis of their composition and physicochemical characteristics.^{82,83} This knowledge, which informs and simplifies hazard testing for dissolved chemicals, is rarely available for MNs, suggesting that fate and exposure testing may need to be incorporated into hazard testing guidance for MNs. For example, the environmental relevance of testing the aquatic toxicity of MNs that rapidly settle out of suspension with pelagic organisms was debated during the workshop. The ongoing efforts at OECD to develop TGs and a GD on MN dissolution, dispersion stability, and environmental fate will inform these decisions, while the TG on MN sorption to activated sludge that is also currently under development will

enable more realistic estimates of surface water and terrestrial nanomaterial concentrations. At a minimum, the toxicity of the corresponding dissolved bulk material (if available) should be determined for a complete interpretation of aquatic hazard data generated for MNs.⁸⁴

Limit Testing. While the concept of limit testing is described in many OECD TGs, its applicability to MNs was not explicitly discussed during the workshop. The use of limit testing to assess the hazard of MNs is complicated by many of the exposure issues described here for concentration–response (multiple exposure concentration) testing. Limit tests employ a recommended maximum exposure concentration to determine whether a substance has hazard potential within reasonable limits. The goal is to identify a single high concentration of the test substance at which no effects are observed, eliminating the need for further testing. OECD TGs 218 and 219 (sediment-water Chironomid testing with spiked sediment⁸⁵ or water⁸⁶) describe the limit-test concentration as “...sufficiently high to enable decision makers to exclude possible toxic effects of the substance, and the limit is set at a concentration which is not expected to appear in any situation.” OECD 218 sets this concentration at or below 1000 mg/kg of sediment. Applicable aquatic TGs^{93,101,130} recommend limit tests be set at 100 mg L⁻¹ (or the highest soluble concentration, whichever is lower) for water-only tests. For substances that form stable dispersions, an existing OECD GD²⁷ (that does not specifically consider MNs) recommends a limit concentration of 1000 mg L⁻¹ or the dispersibility limit, whichever is lower. The application of limit testing based solely on mass concentration is potentially problematic for MNs, as the particle number concentration and surface area vary significantly for a given mass of material present at mean sizes between 1 and 100 nm. Other issues include varying MN transformation rates (i.e., dissolution, agglomeration) at different concentrations and the potential for nanomaterial atypical dose–response curves.

Potential Modifications to Test Procedures. *Adjusting Medium Composition.* A number of potential modifications to standard testing were considered for MN ecotoxicity testing to address the behaviors of MNs described above. One of these modifications is to prescribe a single test medium for each commonly used test organism for use with each bioassay method. Current TGs typically allow for flexibility in selection of the bioassay medium in recognition of variability among various testing facilities. However, for MNs this flexibility can lead to difficulty in comparing test results and potentially a lack of agreement among laboratories that are using the same basic test method. Diluting the test medium (i.e., reducing the ionic strength) or adjusting the pH of the medium away from the point of zero charge of the MN may reduce the rate of agglomeration and settling for many MNs⁸⁷ but may be physiologically stressful for test organisms.⁸⁸ Thus, in selecting the standard test medium, there is a potential trade-off between maintaining organism health and vitality and minimizing the MN agglomeration and transformation rates. For example, *Daphnia magna* growth and reproduction are typically raised with greater water hardness,⁸⁹ but this leads to greater rates of MN agglomeration for charge-stabilized MNs, resulting in lower or less consistent exposure. Choosing an alternate daphnid test species adapted to softer waters (e.g., *Daphnia pulex*⁸⁸) may be a viable alternative. Any modifications to the standard methodology that may alter the physiological stress responses of the test organism should be validated with a positive control experiment such as a reference toxicant test,

which can be found in OECD method validation studies and the open literature.¹²⁹ In addition, some MNs may yield acceptable assay variability in standard test media, and altering standard and historically used test media would limit relative comparisons to previous data generated using OECD ecotoxicity TGs. For MNs where dissolved metal ions may impact the toxicity (e.g., ZnO and AgNPs^{17,29}), it is important to exclude metal chelators such as EDTA as described in previous OECD documents for metal toxicity testing (e.g., algae testing⁹⁰). While some studies have used chelators such as cysteine to eliminate the impact of released ions to highlight the impact of an MN itself, interactions between the chelators and the MN surface may impact MN behaviors and transformations.^{91,92}

Standardizing Test Vessels and Systems. The selection of test vessels can also impact ecotoxicological results.^{93–95} Increasing the consistency of the test vessel dimensions (material, size, aspect ratio, internal surface area) for each test type and species is expected to reduce differences in the rate of MN agglomeration, settling, dissolution, or sorption, although it should be considered that a single type of test vessel may not always be suitable for all types of MNs. A consistent test vessel for each test type and species should be selected from common commercially available products. Assay-specific modifications should also be considered, such as the impact of the agitating mode for the algae test on MN behaviors and the grazing on the bottom of the vessel for the *Daphnia magna* test.^{90,96,97} Furthermore, interlaboratory comparison testing can be used to evaluate specific TG accuracy and precision among laboratories.^{98–100}

Preparing Initial MN Dispersions. There are multiple approaches for preparing MN dispersions for aquatic toxicity testing, such as the use of deionized (DI) water stock dispersions for spiking test media, sonication of MNs in test media, and the use of stabilizing agents. The approaches described in this section relate to the preparation of dispersions in DI water prior to mixing with the test medium. It is often easier to produce stable dispersions of MNs in DI water as a result of the lower ionic strength and thus reduced agglomeration and settling rates. There are several potential approaches to disperse MNs in DI water that can be used individually or in combination: (1) use of commercial dispersants, capping agents, or solvents; (2) use of NOM; and (3) sonication of unmodified MNs.

Many MNs are not stable in aqueous media in the absence of surface coatings or dispersants. When commercial MNs are synthesized with a dispersant or capping agent, it should be considered an integral part of the MN; control experiments can be conducted if it is important to elucidate the impact (stimulatory or inhibitory) of the dispersant or capping agent on the assay results.²⁹ Workshop participants discouraged use of additional synthetic organic solvents or dispersing agents, such as tetrahydrofuran (THF) or sodium dodecyl sulfate (SDS), when dispersing MNs because of their high potential to confound the results, as thoroughly discussed in previous papers.^{12,19,101–103} However, if commercial products use synthetic solvents or dispersing agents in the MN formulation, then the bioassay should be conducted with the product as produced.⁶³ Thus, in these cases carefully designed control experiments (as described in ref 29) are needed to elucidate the toxicity mechanism and avoid artifacts.

Ubiquitous natural dispersants such as NOM may be considered with the recognition of their potential to

significantly alter the MN dispersion stability and toxicity.^{31,32,67,104,105} Environmentally relevant concentrations should be considered;^{106,107} however, to maintain a conservative approach for hazard assessment, only the lowest concentration necessary to achieve a stable dispersion should be used. Workshop participants discussed whether a standard NOM could be identified or used, but no consensus was reached. However, it was agreed that control experiments are essential for understanding the influence of NOM on toxicity. This topic and discussion are covered in greater detail in the [Supporting Information](#). Guidance on evaluating the effects of NOM on polymer toxicity²⁷ and an existing U.S. EPA guideline¹⁰⁸ may be of use in addressing this issue for MN.

Dispersion by sonication is implemented in the OECD TG on MN dispersibility and dispersion stability that is under development, but sonication is known to generate oxidative species in solution as well as pyrolysis conditions. A variety of sonicator types and models exist and differ in power transformation efficiency and in the way in which the energy is delivered to the sample (e.g., sonication probes, bath sonication, and cup-horn sonication). The potential effect of sonication on the MN surface chemistry and size should be evaluated, as this procedure has been shown to destroy or damage CNTs^{109,110} if an ice–water bath is not used. Importantly, sonication may degrade molecules coating MNs,¹¹¹ and in some cases, the sonication process may alter the toxicity of surface coatings^{29,112} or add metal contamination through disintegration of the sonicator tip.¹¹³ However, sonication may provide only short-term dispersion of some MNs, as agglomeration may reoccur after sonication ceases and during the bioassay.

Different approaches exist for dosing test media with MNs, such as creating a working stock dispersion for spiking test media and performing a serial dilution to create test concentrations or direct addition of the test substance to the medium to individually prepare each test concentration. If the agglomerate state of the MNs is not impacted by serial dilution, the stock dispersion approach may be appropriate; if the state of the MNs is impacted by dilution, individual preparation of each concentration should be considered. While the approaches described thus far relate to the production of a stock MN dispersion, it may be advisable to follow a different approach if an MN has more than one potentially toxic component. This approach, which is typically used for testing of chemical mixtures because the various components may be present at different ratios at different concentrations, involves the preparation of a separate dispersion for each concentration.²⁷ One example of MNs with multiple toxic components is CNTs that release toxic metals from the residual metal catalysts. If a stock dispersion is made, the concentration of released metal impurities will be higher in the stock dispersion because dispersed and settled CNTs will both release toxic metals. Dilutions made from the stock dispersion to obtain different dispersed CNT concentrations would have different CNT to metal ion ratios than if a separate dispersion was made for each concentration. If the primary toxic effect is driven by the dissolved metal impurity, a dilution series prepared from this stock dispersion may produce an acceptable dose–response curve; however, the effect may be erroneously attributed to the CNT rather than the impurity. Preparing separate dispersions for each test concentration helps to distinguish effects due to the MN from those due to impurities. However, preparing separate dispersions at low concentrations (<1 mg L⁻¹) could

lead to higher variability in assay results due to the inaccuracy of weighing small masses.

Preparing Dispersions in Assay Chambers for Organism Exposure. After stock dispersions or dispersions for each test concentration are prepared using the procedures described in the proceeding section, it may be necessary to add the dispersions to the test medium. If the dispersibility and dispersion stability TG is used to prepare the dispersion, it is important to note that the TG is designed to test the stability of MNs in different aquatic media and not to prepare the best dispersion for ecotoxicity testing using other OECD methods.

After the addition of dispersed MNs to the test medium, there are multiple options regarding when to test the ecotoxicity of the resulting suspension. One approach is to immediately add the dispersed MN to the test medium. This approach may minimize the variability among laboratories in the initial MN dispersion to which the organisms are exposed if the dispersion procedure is robust. However, the MN settling rate during the course of the ecotoxicity assay may be quite variable as a result of factors such as different test media.

An alternative option for unstable MNs is to first add the dispersed MN to the test medium or to sonicate the sample in the test medium and then to monitor the MN suspension stability over time to determine whether, and wait until, a pseudosteady state is established, at which point the settling rate has reached a minimum (or acceptable level) or there is no longer any detectable settling.²⁷ The MN suspension that has reached a pseudosteady state could be transferred to test vials to start the bioassay. However, no consensus was reached in the workshop on a recommended maximum time limit to reach the pseudosteady state. Measurements may be needed to assess whether transferring the suspension causes additional agglomeration, settling, and sorption to test containers, resulting in reduced exposure. Settled material included in bioassays may also act as a source of dissolved materials or resuspended particles and potentially alter the system chemistry (e.g., oxidation or reduction states).¹¹⁴ The approach described above is conceptually similar to water-accommodated fraction (WAF) methods frequently used in petroleum testing.^{28,115,116} Some similarities are that energy is first added to the system (e.g., by sonication for MNs and by blender mixing or slow stirring for petroleum) followed by a period of settling for MNs or separation for petroleum and then collection of the MN dispersion or WAF, leaving behind the unsuspended material. In both cases, the goal is to produce repeatable water column exposures. However, in both cases, physical effects or continued release of toxic components from the separated material are excluded from the hazard assessment. For example, physical effects of petroleum can be significant in oil spills, and Park et al.¹¹⁷ demonstrated that removal of settled particles reduced the toxicity of Ag MNs to *D. magna* but not *Oryzias latipes*. Due in part to the many uncertainties associated with this approach, a consensus was not reached on the application of WAF approaches for MN hazard testing. However, it was noted that WAF approaches are suggested for some difficult-to-test substances in existing guidance documents.²⁶

Potential MN Artifacts. When testing the potential ecotoxicological effects of MNs, a significant complication is that the MNs themselves may cause artifacts or misinterpretations in ecotoxicology assays.^{29,118–120} A comprehensive discussion of the potential artifacts and misinterpretations inherent in bioassay testing of MNs is provided in a recent publication²⁹ and is beyond the scope of this review. Briefly,

Table 1. Arguments for and against Implementing the $\pm 20\%$ Test Specification for Aquatic Bioassays Testing Nanomaterials That Are Not Inherently Stable in Bioassay Test Media; It Was Generally Agreed That Attempts Should Be Made To Maintain the Concentration

advantages of the 20% test specification	challenges related to applying the 20% test specification with MNs
Maintaining high and stable concentrations of nanomaterials will lead to more reproducible test results and agreement among laboratories.	Attempting to maintain stable concentrations of MNs that are inherently unstable in water lowers the environmental relevance and does not account for MN transformation. The worst-case scenario is not achieved if the transformation product is more toxic than the parent material (e.g., dissolution of metals). It is generally not recommended that the toxicity of a parent material be tested if its half-life is less than 12 h. ²⁶
Maintaining relatively stable exposure concentrations is consistent with the existing risk paradigm of assessing hazard independently from exposure. In this paradigm, hazard values are often interpreted in context with natural factors that affect fate and exposure.	It is difficult or impossible to maintain the stability of nanomaterials that are not stable in test media. Even if the concentration is maintained, the state of agglomeration and/or dissolution of the particles would likely change. The use of dispersants that would assist in maintaining the stability is generally not favored. ^{9,26}
Maintaining stable concentrations facilitates the calculation of toxicity end points without the need for weighted averages (or other methods).	Additional logistics added to maintain the stability of unstable MNs (e.g., frequent water exchanges, flow-through conditions, agitation) are more labor-intensive and expensive, are not tailored to particle delivery (e.g., clogging of tubing), and may result in repeated tests and increased costs. Water-accommodated fraction approaches are already recommended for difficult-to-test substances such as partially miscible petroleum products. ²⁶ This involves testing of the stabilized fraction that is more relevant to water column testing; testing of the stabilized fraction is expected to allow for a more consistent exposure concentration and thus should better facilitate the calculation of end points. Excluding settled particles from bioassays may reduce the variability by avoiding confounding physical effects. However, excluding the settled particles may remove the physical effects and may not facilitate a worst-case determination of the toxicity.

issues such as the use of control experiments, evaluation of nutrient depletion caused by MNs, interference of the MN with the assay measurement (e.g., algal density), and inaccurate dosimetry quantification and metrics need attention in order to achieve consistent toxicological results. MNs may confound toxicity measurements by limiting the applicability of common approaches. For example, a recent study showed that Coulter counter and hemocytometer measurements of algal density after exposure to titanium dioxide or gold nanoparticles were impeded as a result of heteroagglomeration between the algae cells and the MN; fluorometric methods were found to be the most suitable.¹¹⁹ Overall, multiple methods (e.g., Coulter counter and fluorometric analysis of algae), ideally using promulgated or standard test methods, should be utilized when available and careful consideration of relevant control experiments is critical.

■ CONSIDERATIONS FOR APPLYING THE 20% EXPOSURE SPECIFICATION TO TESTING OF MNS

OECD harmonized aquatic toxicity TGs discuss acceptable limits of variation in water column concentrations and provide suggestions for approaches to maintain these limits. These are invariably set at 80% to 120% of the nominal or initial (immediately upon dosing) measured water column concentrations. The TGs vary in specifying whether changes in water column concentration should be relative to nominal or measured values. Further, TGs vary in their prescription of what should be done if the 20% exposure specification is exceeded. In some TGs, this outcome simply determines whether exposure–response analyses and reporting can be based on nominal rather than measured concentration values.^{97,121} In others, the need for more frequent substance quantification is discussed,^{90,122} but neither a specific schedule for these analyses nor an approach to determine the rate of concentration change is provided. In other TGs, it is suggested that the exposure system be preconditioned (to limit adsorption), medium renewal intervals be shortened, or continuous renewal (or flow-through) systems be employed. It seems implicit in the TGs that variation in excess of $\pm 20\%$ does not constitute test failure as long as diligent efforts were made to attempt to maintain consistent exposure, the exposure was quantified on the basis of measured values, and

measurements were made frequently during a test or medium renewal period. Beyond the TGs, there are documents^{27,123} that provide some guidance when the 20% exposure specification is exceeded. These GDs state that if the concentrations remain within $\pm 20\%$ then the results may be based on nominal or mean measured values and that if the concentrations deviate by more than $\pm 20\%$ then the results must be reported on the basis of measured values (geometric or time-weighted mean). It is also important to recognize that among these TGs and GDs, substance losses are generally attributed to their elimination from test systems (e.g., by volatilization and chemical degradation processes). In TGs and GDs where substance losses from the water column (but not from the test system) are observed (e.g., by settling or physical separation), it is recommended that insoluble components be removed by filtration, centrifugation, or other separation methods;^{26,27} this is potentially applicable to MNs on a case-specific basis to ensure that the worst-case, most conservative hazard result is generated, but consensus on this approach was not reached by the workshop participants.

Some advantages and disadvantages of the $\pm 20\%$ exposure specification are summarized in Table 1. On the basis of the literature and the experience of workshop participants, it was concluded that for many MNs, maintaining water column concentrations within $\pm 20\%$ of the initial concentration during ecotoxicity assays with or without medium renewal and without the use of dispersants or solvents is likely to be difficult if not logistically infeasible, especially at higher concentrations (e.g., mg L⁻¹). Even if a stable dispersion is initially prepared, it may not be possible to maintain consistent exposure if changes in the state of agglomeration, particle dissolution, and/or some other transformation of the particles continue to occur during the bioassay. Examples of rapid decreases in MN concentration and increases in agglomeration are shown in Figure 1. Clearly, it is important to consider whether the 20% exposure specification should be applied to MNs, and this suggests a need for guidance on how MN losses should be addressed and reported. Unfortunately, it is unclear from TGs what the basis or rationale for setting the level at $\pm 20\%$ is, other than the goals of maintaining stable exposures, facilitating end-point calculation, and avoiding overlapping exposure concentrations among treatment levels within a concentration series. Hence,

it is difficult to assess whether this exposure specification would be more or less applicable to MNs compared with soluble chemicals. Regardless of the specific level of acceptable change in the aqueous concentration, the critical issue is how the MN concentration (and other metrics such as particle size, particle count, or surface) should be quantified during testing. Approaches for calculating toxicity end points if there is a greater than 20% decrease in the aqueous-phase concentration are discussed in the [Supporting Information](#).

DOSIMETRY AND INTERPRETATION

Dosimetry. An inherent hypothesis in nanotoxicology is that the size-specific properties that make MNs useful for technology applications will also be important for determining biological effects.^{39,124–131} However, a consensus on the particle-specific or unique effects that consistently apply to specific classes of MNs has yet to be reached.^{132,133} Various studies in the ecotoxicology literature have reported higher toxicities for smaller particles,^{134–137} although size-related toxicity is not always observed.^{138,139} It is widely recognized that the standard mass-only dose metric paradigm used in toxicology for traditional substances may not adequately represent exposure–response relationships for MNs.^{39,140,141} The mass-only paradigm is further compromised by decreasing suspended MN concentrations during bioassays, a scenario where a time-weighted averaging approach more accurately reflects exposure concentrations but is seldom used in practice. There are numerous alternative dose metrics for MNs other than mass; the most commonly discussed are total available particle surface area and particle number concentration.¹⁴⁰ For example, Van Hoecke and co-workers^{142,143} reported that the available surface area ($\text{m}^2 \text{L}^{-1}$) of CeO_2 and SiO_2 MNs was better correlated with growth inhibition of algal cells than was the mass concentration. For some soluble metal MNs (e.g., Ag, Cu), the dissolved fraction (and dissolution kinetics) in test media also must be considered in dosimetry determinations.^{137,144–146} While some studies have reported that the toxicological response is correlated with certain MN properties, it has been difficult to confirm these trends across toxicological investigations. This is likely in part a result of poor understanding of how the state of MN exposure differs (e.g., different states of polydispersity) among investigations because of challenges associated with measuring polydisperse MN suspensions in test media. Furthermore, size-unique effects are suggested to be most likely to occur below 30 nm,¹⁴⁷ and therefore, studies focusing on size-related effects above 30 nm may not isolate particle-specific effects.

The aerosol science literature has addressed alternative dose metrics for particles (e.g., see refs 148–151), and several recent ecotoxicology studies have reported improved dose–response expression by surface area,^{134–136,152} ion release,^{136,152} or particle number.¹⁵³ However, the development of a standardized alternative dose metric for MNs for hazard assessments is encumbered for a number of reasons: (1) it is unlikely that any one alternative dose metric will provide an improvement over mass for all MNs in all test systems; (2) it is more difficult to directly measure surface areas and particle numbers compared with mass concentrations at bioassay-relevant concentrations in bioassay media,¹⁴⁰ although methods are becoming available;⁵⁹ (3) unless size distribution data are known or measurable, polydisperse particle suspensions in test media will further complicate the interpretation of exposure relative to effect; and (4) dynamic changes in dispersion

stability or consistency (suspended concentration, agglomeration, and dissolution) confound concise interpretation and render dose metric conversions from size and mass less accurate. Unless the particle number concentration and/or size distribution are directly measured,⁵⁹ the uncertainty in the surface area and MN number concentrations will be substantially higher than those based on mass concentrations. In this context, OECD recommended that particle number, surface area, and mass should all be measured when feasible to allow calculation of alternative dose metrics.²³ These measurements should be monitored throughout the test at all test concentrations to account for concentration-specific changes in dispersion characteristics.

Interpretation. Bioassays involving exposure to suspended MNs need to be interpreted on the basis of multiple factors: their relevance and appropriateness for assessing the tested MN, the consistency of the exposure (stable concentration, agglomeration, and dissolution), whether maintaining a consistent exposure is possible in the bioassay-method-specific test system, the accuracy of the representation of the exposure (e.g., whether the frequency of characterization measurements was sufficient to capture changes in exposure during the bioassay), whether nanospecific bioassay acceptability criteria (e.g., sufficiently consistent exposure concentration with respect to agglomeration and dissolution) are met, and whether the characterization and monitoring data during the bioassay are amenable to expressing the data in terms of an alternative dose metric. If the suspended MNs cannot be maintained within 20% of the starting value within the water phase (with respect to concentration, agglomeration, and dissolved fraction), it is difficult to employ any dose metric without complicated and potentially inconsistent conversions,¹⁵⁰ and a time-weighted mass approach may be a more expedient option to express dosimetry. While challenging calculations may be feasible in research, a more straightforward approach is needed for hazard and risk assessments. However, most of the historical literature used to determine regulatory hazard concerns for chemicals are mass-based and provide a critical benchmark against which to compare the toxicity of new MNs.

SEDIMENT TESTING

Many of the considerations previously discussed for water column testing are relevant to sediment tests, with the notable exception that there is no need to remove insoluble test material according to standard assay protocols.^{85,154} While the latter is a major conceptual difference between tests of MN and traditional chemicals with pelagic organisms, it is not an issue in sediment testing. Some added complications are that MN interactions in sediments can significantly alter the MN properties, and methods for quantifying concentration or other MN characteristics in sediments are very limited. However, in view of the fact that most MN suspensions are generally not stable in environmentally relevant water chemistries ([Figure 1](#)), there was consensus from the expert workshop that consideration of sediment exposure and hazard is relevant and in many cases more representative of environmental exposure than aqueous tests. Current sediment toxicity standard methods acknowledge significant uncertainty regarding test substance homogeneity, exposure, bioavailability, and synergisms. Thus, poorly understood bioavailability issues are commonplace in sediment testing and are not unique to nanoecotoxicology. An evaluation of available standardized sediment bioassay methods (OECD, EPA, ASTM, etc.)

Table 2. Summary of Major Issues Discussed by Workshop Participants Where Consensus Was Reached or Was Not Reached and Research Recommendations To Fill Knowledge Gaps That Prevented Consensus

issue	consensus items from the workshop	items lacking consensus	key research recommendations to address items lacking consensus
Feasibility of considering hazard and exposure separately for MNs	The focus of the guidance document is to increase the consistency of bioassay results used for hazard assessment. However, dispersion stability must be considered in bioassay method selection and monitoring. Effort should be made to maintain a consistent MN concentration when logistically feasible.	Designating a limit of acceptable exposure variability either at 20% (the $\pm 20\%$ test specification) or some other level over the duration of the bioassay.	Approaches for maintaining MNs in suspension (e.g., frequent medium renewal, flow-through delivery, and test medium modifications) should be studied. Testing of flow-through systems should consider the potential for increased MN concentrations in the test system resulting from settled material not removed from chambers. It should be determined whether maintaining stable concentrations reduces variability in test results when agglomeration and dissolution cannot be avoided. Time-weighted averaging and more complex approaches to express variable exposures should be investigated. The extent to which settled MNs influence ecotoxicity results should be determined. Research could also focus more broadly on quantifying the uncertainties that arise when exposure varies beyond specific thresholds (including $\pm 20\%$).
Dispersion methods	It is acceptable to disperse MNs in either working stocks (for spiking biological media) or to disperse MNs directly in the test media. Working stocks should be used only if there is a single substance in the MN that exerts toxicity. The optimal method will be contingent on target concentration, medium, and bioassay method selection.		
Addition of substances to enhance MN dispersion	Dispersants should not be used to prepare nanomaterial suspensions for biological testing unless they are present in the (commercial) product formulation. Natural organic matter (e.g., humic acid) may be used as a dispersant; however, control experiments are essential to understand the influence of NOM on toxicity. ⁹	The type of natural organic matter to recommend.	Impacts of different types of natural organic matter on MN stability and toxicity testing results should be investigated.
Modifications of methods to address MN instability	Water column bioassays should be conducted to maintain consistency with chemical hazard assessment practices. However, alternative water column bioassay designs should be considered for very unstable MNs.	Whether to allow particle agglomeration, settling, and dissolution kinetics to come to equilibrium before adding test organisms. It was agreed this could be presented as an option for nondispersible materials along with caveats. Whether effects such as inducing turbulence and flow-through systems should be employed to maintain particle concentration.	The reproducibility of test results when initial suspensions versus pseudosteady-state suspensions are tested, the relative impact of chemical versus physical effects on MN toxicity, and the impact of approaches (turbulence and flow-through systems) to maintain particle concentration on MN toxicity should be assessed.
Standard test media and test chambers	One standard exposure chamber and test medium for each OECD test method/organism should be recommended for MNs to maximize test consistency. If the test medium is modified (relative to current practice), a positive control test with a reference toxicant in the modified medium is recommended.	Whether it is acceptable to modify standard media to increase particle stability and ultimately maintain MN concentration. pH adjustments away from the isoelectric point (within biological limits) are more acceptable. However, there was concern that ionic strength dilutions could impact animal health and decrease comparability with historic data sets.	Research to support the development of a single test medium for each TG that would lead to the most reliable ecotoxicity results for MN testing should be carried out. Studies should quantify acceptable thresholds for maintaining organism health and environmental relevance. Different types of test containers (size, type of material, geometry) should be tested to assess the robustness of the different TGs with regard to this parameter. The impact of the agitating medium should be evaluated for tests requiring agitation, such as the algae growth inhibition test. ³⁰ While using standard exposure chambers may increase hazard data consistency, the utility of chamber modifications for the purpose of environmental risk assessment needs further consideration.
Expressing and interpreting dosimetry	Preliminary testing is recommended to determine particle stability in the specific test system and biological test medium prior to organism testing to inform test design, characterize the monitoring frequency, and reduce animal use by reducing the number of unsuccessful or unacceptable tests.	Establishing a standard dose metric and reliable analytical techniques for monitoring MNs. Without readily available direct measurement methods, it will be difficult to relate dose response to surface area or particle number metrics for heterodispersed suspensions of MNs that are unstable in biological media over time.	It is important to develop, validate, and standardize analytical methods to directly measure particle number concentrations and size distributions in aqueous samples at toxicologically relevant concentrations (sometimes low $\mu\text{g L}^{-1}$). Best practices for calculating exposure—response values also need to be developed.
Sediment toxicity testing	Sediment toxicity tests are most relevant for MNs that are unstable in the medium.	Whether very unstable MNs should be tested only in sediments (i.e., no water column testing).	Characterization methods for particles in the complex sediment matrix should be developed, especially for carbon-based MNs. For metal and metal oxide MNs, the development of methods to differentiate between MNs, dissolved metal ions, and MN agglomerates is needed. Dosing directly to the sediment versus indirectly dosing the sediment through the overlying water (for a surficial sediment exposure) and the associated impacts homogeneity and toxicological results should be investigated.

suggested that the test end points assessed in these methods will contribute valuable MN hazard information.¹³ While it may not be currently feasible to rigorously characterize many types of MNs present in sediment, the consistency of sediment toxicity bioassays can still be generally improved by implementing standards for particle preparation, dispersion, spiking, and equilibration in sediment.¹¹ Further, the use of a standardized (e.g., OECD) freshwater sediment in MN spiking studies would reduce variability in bioassay results relative to the use of field-collected sediments because sediment-specific factors (e.g., organic carbon concentration) that can influence toxicity assay results are controlled. This discussion is divided into different important topics for MN sediment toxicity testing: (1) methods for consistently spiking sediment, (2) equilibration time, and (3) sampling and analysis of MNs in sediments during and after the test.

Methods for Spiking and Determining Homogenization. Spiking of aquatic sediments is generally expected to be more consistent in terms of homogeneity if the materials are predisposed into relevant water according to standardized methods rather than adding dry MNs to sediment.^{12,23} This is related to general difficulties regarding homogenizing chemicals into sediments.¹⁵⁵ If a MN is added to sediment in powder form (undispersed), it is likely that substantial clumping of particles within the sediment would occur, resulting in greater heterogeneity and therefore greater variability between bioassay test replicates.¹¹

As previously discussed, the use of a standardized sediment in MN spiking studies would likely lead to more comparable results than the use of field-collected sediments. Two alternative MN spiking methods have been discussed and used for sediment MN toxicity testing: (1) direct addition of dispersed MNs to the sediment followed by homogenization^{37,156,157} and (2) indirect addition of MNs to the overlying water, followed by subsequent settling of the MN to the surficial sediment.^{12,158,159} In the literature, the direct addition method is much more frequently used. Selection of one (or both) of these methods may relate to the test objectives, study system, or functional ecology of the organism used in the test or at the site of concern. For instance, a testing laboratory may elect to use the direct addition method for an infaunal deposit-feeding organism, which will feed on sediment below the sediment surface, while the indirect method may be desirable for an epibenthic surface-deposit-feeding or filter-feeding organism, which will interact to a substantially larger degree with the sediment directly below the water–sediment interface. Research is needed to determine how to most consistently spike sediments (e.g., mixing method, duration) by these two spiking strategies so that particles are dispersed throughout the sediment as homogeneously as practical to increase the inter-replicate reliability. Additionally, research is needed to better understand how water exchanges, which are typically performed during longer-term sediment toxicity tests, may impact MN concentrations and distributions within or on the surface of the sediment.

Equilibration Time To Reach a Pseudosteady State after Spiking with MNs. It is well-known that the time required to reach a quasi-steady state by equilibrium partitioning for spiked sediment studies is important for determining bioavailability, especially for hydrophobic compounds that take a long time period (weeks to months) to approach pseudoequilibrium in sediments.¹⁵⁵ Thus, 2 weeks¹⁶⁰ to 4 weeks^{161,162} on a roller mill is a typical equilibration time

to allow interactions between the spiked compound and ligands to approach some level of steady state. However, currently available OECD sediment spiking methods recommend 48 h of equilibration.^{85,154,163} As reflected by recommended ASTM and EPA equilibration mixing times, a 48 h duration, while convenient, does not allow adequate equilibration-reaction of metals in spiked sediment¹⁶⁴ but may provide a worst-case scenario in terms of greater MN bioavailability. While selection of equilibration times may be contingent on experimental objectives, research is needed to determine how interactions of MNs with sediment may change over time in order to determine the optimal equilibration time prior to test organism addition and exposure.

Sampling and Analysis. While current gaps in methods for MN characterization may limit the determination of particle characteristics following spiking into sediment, certain measurements may still be performed, such as the use of ICP-MS to determine the total elemental concentration for metal and metal oxide MNs. It is practical to take samples for such measurements from the whole sediment, sediment porewater, and overlying water at test initiation and termination, as recommended in current OECD sediment testing guidance; however, MN-specific modifications of porewater separation methods may be needed to yield accurate results.

■ WORKSHOP FINDINGS

While the findings discussed in this workshop primarily pertain to issues related to the applicability of OECD aquatic toxicity TGs, many of the findings also more widely apply to test methods for other documentary standards agencies (e.g., ISO and ASTM) and for terrestrial organism testing, academic research, and regulatory decisions. The discussion of the workshop participants led to both convergent and divergent opinions on how the major issues impacting the consistency, environmental relevance, and accuracy of aquatic bioassay results should be handled in aquatic toxicity testing. To the extent possible, it is desirable to minimize the amount of developmental work performed by commercial testing companies, such as assessing which procedure should be used to disperse MNs in the test medium or designing a complicated system to comply with the $\pm 20\%$ test specification. A summary of issues for which workshop participants both achieved and failed to achieve consensus is summarized in Table 2; where consensus was not achieved, targeted research studies are recommended in the table. The proposed research is designed to support the development of precise guidance for conducting OECD aquatic toxicity tests that will simplify this process for commercial testing laboratories and to help regulators interpret the results through the OECD aquatic toxicity testing GD to be developed following this paper.

The workshop participants agreed that it can be acceptable to disperse particles in either working stocks (for spiking test media) or dispersing MNs directly into test media, as described above. The optimal method will be contingent on the physicochemical properties of the MN, the target concentration, the medium, and the bioassay method selected, and preliminary data should be gathered prior to decision making. Synthetic dispersants should not be used to prepare MN suspensions for aquatic toxicity testing; however, if they are part of the (commercial) product formulation, then the bioassay should be conducted with the as-produced material. This recommendation aligns with previous aquatic toxicity test guidances.^{26,123,165} Natural dispersants such as dissolved

organic carbon (i.e., humic acid) may be relevant, but their impact on the toxicity of MNs should be considered (e.g., for metal MNs); the total organic carbon concentration should be within the range of surface waters. Additionally, while particle stability is likely to be an issue, water column bioassays should be conducted with the goal of maintaining exposure consistency to abide by chemical hazard assessment practices (e.g., REACH²⁸). However, alternative water column bioassay designs or sediment exposures should be considered for very unstable MNs, adapting guidance described in the difficult substances document.²⁷ For aquatic toxicity bioassays with MNs, an exposure chamber with consistent dimensions and one test medium for each OECD test method/organism is desirable for MNs to increase test consistency. Standard testing end points and numbers of test replicates should be applicable to MN testing. Some preliminary but nonexhaustive experimentation to determine particle stability in the test medium prior to organism testing would be informative for test design and reducing animal use in unsuccessful tests.

While the workshop participants did not come to consensus on whether the 20% test specification in the water column can be consistently applied for MNs, the group agreed that an effort should be made to maintain the concentration when logistically feasible. Consensus was not reached on whether turbulence or flow-through systems should be employed to maintain the particle concentration. Also, no consensus was reached on whether to allow particle agglomeration, settling, and dissolution kinetics to come to equilibrium before adding test organisms, as related to WAF testing. While some workshop participants agreed that a pseudosteady state (or constant concentration) was likely to lead to greater test reliability and repeatability, there were divergent opinions on allowing a pseudosteady state to be reached and removal of the settled fraction of particles, as they may not offer a worst-case scenario; it should be noted that a pseudosteady state may not occur in the aqueous phase for some MNs (e.g., complete settling from suspension or continual ion release due to adsorption to container or ligand surfaces). No consensus was reached on whether altering standard media to increase the particle stability and ultimately maintain the concentration was acceptable. While pH adjustments away from the isoelectric point (within biological limits) were generally more acceptable, there was concern that ionic strength dilutions would impact animal health and decrease comparability with historic data sets. While consensus was not reached on these items, suggestions for future research to help resolve the lack of consensus are provided in Table 2. Additional suggestions for future research to support more definitive suggestions for modifications to OECD aquatic toxicity test methods are provided in Table S1 in the Supporting Information; the research topics in Table S1 are categorized by section of the review, while those in Table 2 are provided for each area for which consensus was not reached.

Following the consensus in Table 2 will help to substantially improve the reliability and data quality of nanoecotoxicology research and provide substantive improvements for regulatory testing. Facilitating the aquatic toxicity testing of MNs using standardized methods will help MN risk assessments to be conducted more efficiently. This will potentially allow MN-enabled products to reach the market in a shorter time period, allow registrants to improve the quality of data for fulfilling regulative information requirements, and promote green product design by identifying MNs with potentially significant

toxicological effects or with the potential to design more benign alternatives early in the development stages.

■ ASSOCIATED CONTENT

● Supporting Information

Supplemental discussions of definitions and measurements of “dissolved” substances, which type of NOM to recommend for aquatic toxicity testing, and the impact of calculating toxicity end points where the 20% specification is not achievable and a table describing key additional research topics for each section of this review. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b00997.

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Notes

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■ REFERENCES

- (1) Bowman, D. M.; Hodge, G. A. A small matter of regulation: an international review of nanotechnology regulation. *Columbia. Sci. Technol. Law Rev.* **2007**, *VIII*, 1–36.
- (2) Maynard, A.; Bowman, D.; Hodge, G. The problem of regulating sophisticated materials. *Nat. Mater.* **2011**, *10* (8), 554–557.

- (3) Beaudrie, C. E. H.; Kandlikar, M.; Satterfield, T. From cradle-to-grave at the nanoscale: gaps in U.S. regulatory oversight along the nanomaterial life cycle. *Environ. Sci. Technol.* **2013**, *47* (11), 5524–5534.
- (4) Hansen, S. F. Regulation and risk assessment of nanomaterials—too little, too late. Ph.D. Dissertation, Technical University of Denmark, Kongens Lyngby, Denmark, 2009.
- (5) Grieger, K. D.; Hansen, S. F.; Baun, A. The known unknowns of nanomaterials: Describing and characterizing uncertainty within environmental, health and safety risks. *Nanotoxicology* **2009**, *3* (3), 222–233.
- (6) Kah, M.; Hofmann, T. The Challenge: Carbon nanomaterials in the environment: New threats or wonder materials? *Environ. Toxicol. Chem.* **2015**, *34* (5), 954–954.
- (7) OECD Guidelines for the Testing of Chemicals, Section 2: Effects on Biotic Systems; Organisation for Economic Co-operation and Development: Paris, 2014.
- (8) Crane, M.; Handy, R. D.; Garrod, J.; Owen, R. Ecotoxicity test methods and environmental hazard assessment for engineered nanoparticles. *Ecotoxicology* **2008**, *17* (5), 421–437.
- (9) Handy, R.; van den Brink, N.; Chappell, M.; Mühling, M.; Behra, R.; Dušinská, M.; Simpson, P.; Ahtainen, J.; Jha, A.; Seiter, J.; Bednar, A.; Kennedy, A.; Fernandes, T.; Riediker, M. Practical considerations for conducting ecotoxicity test methods with manufactured nanomaterials: what have we learnt so far? *Ecotoxicology* **2012**, *21* (4), 933–972.
- (10) Kühnel, D.; Nickel, C. The OECD expert meeting on ecotoxicology and environmental fate—Towards the development of improved OECD guidelines for the testing of nanomaterials. *Sci. Total Environ.* **2014**, *472*, 347–353.
- (11) *Ecotoxicology and Environmental Fate of Manufactured Nanomaterials: Test Guidelines*; Organisation for Economic Co-operation and Development: Paris, 2014.
- (12) Handy, R. D.; Cornelis, G.; Fernandes, T.; Tsyusko, O.; Decho, A.; Sabo-Attwood, T.; Metcalfe, C.; Steevens, J. A.; Klaine, S. J.; Koelmans, A. A.; Horne, N. Ecotoxicity test methods for engineered nanomaterials: Practical experiences and recommendations from the bench. *Environ. Toxicol. Chem.* **2012**, *31* (1), 15–31.
- (13) Diamond, S.; Utterback, D.; Andersen, C.; Burgess, R.; Hirano, S.; Ho, K.; Ingersoll, C.; Johnson, M.; Kennedy, A.; Mount, D.; Nichols, J.; Pandorf, P.; Rygielwicz, P.; Scott-Fordsmand, J.; Stewart, K. Review of OECD/OPPTS—Harmonized and OECD Ecotoxicity Test Guidelines for Their Applicability to Manufactured Nanomaterials; U.S. Environmental Protection Agency: Washington, DC, 2009.
- (14) Preliminary Review of OECD Test Guidelines for Their Applicability to Manufactured Nanomaterials; Organisation for Economic Co-operation and Development: Paris, 2009.
- (15) Lowry, G. V.; Gregory, K. B.; Apte, S. C.; Lead, J. R. Transformations of nanomaterials in the environment. *Environ. Sci. Technol.* **2012**, *46* (13), 6893–6899.
- (16) Liu, J.; Hurt, R. H. Ion release kinetics and particle persistence in aqueous nano-silver colloids. *Environ. Sci. Technol.* **2010**, *44* (6), 2169–2175.
- (17) Ma, H.; Williams, P. L.; Diamond, S. A. Ecotoxicity of manufactured ZnO nanoparticles – A review. *Environ. Pollut.* **2013**, *172*, 76–85.
- (18) Petersen, E. J.; Zhang, L.; Mattison, N. T.; O'Carroll, D. M.; Whelton, A. J.; Uddin, N.; Nguyen, T.; Huang, Q.; Henry, T. B.; Holbrook, R. D.; Chen, K. L. Potential release pathways, environmental fate, and ecological risks of carbon nanotubes. *Environ. Sci. Technol.* **2011**, *45* (23), 9837–9856.
- (19) Petersen, E. J.; Lam, T.; Gorham, J. M.; Scott, K. C.; Long, C. J.; Stanley, D.; Sharma, R.; Alexander Liddle, J.; Pellegrin, B.; Nguyen, T. Methods to assess the impact of UV irradiation on the surface chemistry and structure of multiwall carbon nanotube epoxy nanocomposites. *Carbon* **2014**, *69*, 194–205.
- (20) Nowack, B.; David, R. M.; Fissan, H.; Morris, H.; Shatkin, J. A.; Stintz, M.; Zepp, R.; Brouwer, D. Potential release scenarios for carbon nanotubes used in composites. *Environ. Int.* **2013**, *59*, 1–11.
- (21) Kingston, C.; Zepp, R.; Andrad, A.; Boverhof, D.; Fehir, R.; Hawkins, D.; Roberts, J.; Sayre, P.; Shelton, B.; Sultan, Y.; Vejins, V.; Wohlleben, W. Release characteristics of selected carbon nanotube polymer composites. *Carbon* **2014**, *68*, 33–57.
- (22) European Commission. Commission recommendation of 18 October 2011 on the definition of nanomaterial (text with EEA relevance). *Off. J. Eur. Union, L* **2011**, *275*, 38–40.
- (23) *Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials*; Organisation for Economic Co-operation and Development: Paris, 2012.
- (24) Palmqvist, A.; Baker, L.; Forbes, V.; Gergs, A.; von der Kammer, F.; Luoma, S.; Lützhöft, H.; Salinas, E.; Sorensen, M.; Steevens, J. Nanomaterial environmental risk assessment. *Integr. Environ. Assess. Manage.* **2015**, *11* (2), 333–335.
- (25) Lead, J. R.; Wilkinson, K. J. Environmental Colloids and Particles: Current Knowledge and Future Developments. In *Environmental Colloids and Particles*; John Wiley & Sons: Chichester, U.K., 2007; pp 1–15.
- (26) *Aquatic Toxicity Testing of Sparingly Soluble, Volatile and Unstable Substances*; European Centre for Ecotoxicology and Toxicology of Chemicals: Brussels, Belgium, 1996.
- (27) *Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures*; Organisation for Economic Co-operation and Development: Paris, 2000.
- (28) *Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7b: Endpoint Specific Guidance*; European Chemicals Agency: Helsinki, Finland, 2011.
- (29) Petersen, E. J.; Henry, T. B.; Zhao, J.; MacCuspie, R. I.; Kirschling, T. L.; Dobrovolskaia, M. A.; Hackley, V.; Xing, B.; White, J. C. Identification and avoidance of potential artifacts and misinterpretations in nanomaterial ecotoxicity measurements. *Environ. Sci. Technol.* **2014**, *48* (8), 4226–4246.
- (30) Sijm, D.; Rikken, M.; Rorije, E.; Traas, T.; McLachlan, M.; Peijnenburg, W. Transport, accumulation and transformation processes. In *Risk Assessment of Chemicals*; van Leeuwen, C. J., Vermeire, T. G., Eds.; Springer: Dordrecht, The Netherlands, 2007; pp 73–158.
- (31) Edgington, A. J.; Roberts, A. P.; Taylor, L. M.; Alloy, M. M.; Reppert, J.; Rao, A. M.; Mao, J.; Klaine, S. J. The influence of natural organic matter on the toxicity of multiwalled carbon nanotubes. *Environ. Toxicol. Chem.* **2010**, *29* (11), 2511–2518.
- (32) Edgington, A. J.; Petersen, E. J.; Herzing, A. A.; Podila, R.; Rao, A.; Klaine, S. J. Microscopic investigation of single-wall carbon nanotube uptake by *Daphnia magna*. *Nanotoxicology* **2014**, *8* (S1), 2–10.
- (33) Petersen, E. J.; Pinto, R. A.; Landrum, P. F.; Weber, W. J., Jr. Influence of carbon nanotubes on pyrene bioaccumulation from contaminated soils by earthworms. *Environ. Sci. Technol.* **2009**, *43* (11), 4181–4187.
- (34) Cui, X.; Mayer, P.; Gan, J. Methods to assess bioavailability of hydrophobic organic contaminants: Principles, operations, and limitations. *Environ. Pollut.* **2013**, *172*, 223–234.
- (35) Kukkonen, J. V.; Mitra, S.; Landrum, P. F.; Gossiaux, D. C.; Gunnarsson, J.; Weston, D. The contrasting roles of sedimentary plant-derived carbon and black carbon on sediment-spiked hydrophobic organic contaminant bioavailability to *Diporeia* species and *Lumbriculus variegatus*. *Environ. Toxicol. Chem.* **2005**, *24* (4), 877–885.
- (36) You, J.; Landrum, P. F.; Lydy, M. J. Comparison of chemical approaches for assessing bioavailability of sediment-associated contaminants. *Environ. Sci. Technol.* **2006**, *40* (20), 6348–6353.
- (37) Petersen, E. J.; Huang, Q.; Weber, W. J., Jr. Relevance of octanol–water distribution measurements to the potential ecological uptake of multi-walled carbon nanotubes. *Environ. Toxicol. Chem.* **2010**, *29* (5), 1106–1112.
- (38) Praetorius, A.; Tufenkji, N.; Goss, K.-U.; Scheringer, M.; von der Kammer, F.; Elimelech, M. The road to nowhere: equilibrium partition coefficients for nanoparticles. *Environ. Sci.: Nano* **2014**, *1* (4), 317–323.

- (39) Klaine, S. J.; Alvarez, P. J. J.; Batley, G. E.; Fernandes, T. F.; Handy, R. D.; Lyon, D. Y.; Mahendra, S.; McLaughlin, M. J.; Lead, J. R. Nanomaterials in the environment: Behavior, fate, bioavailability, and effects. *Environ. Toxicol. Chem.* **2008**, *27* (9), 1825–1851.
- (40) Hitchman, A.; Sambrook Smith, G. H.; Ju-Nam, Y.; Sterling, M.; Lead, J. R. The effect of environmentally relevant conditions on PVP stabilised gold nanoparticles. *Chemosphere* **2013**, *90* (2), 410–416.
- (41) Levard, C.; Hotze, E. M.; Colman, B. P.; Dale, A. L.; Truong, L.; Yang, X. Y.; Bone, A. J.; Brown, G. E.; Tanguay, R. L.; Di Giulio, R. T.; Bernhardt, E. S.; Meyer, J. N.; Wiesner, M. R.; Lowry, G. V. Sulfidation of silver nanoparticles: natural antidote to their toxicity. *Environ. Sci. Technol.* **2013**, *47* (23), 13440–13448.
- (42) Levard, C.; Mitra, S.; Yang, T.; Jew, A. D.; Badireddy, A. R.; Lowry, G. V.; Brown, G. E. Effect of chloride on the dissolution rate of silver nanoparticles and toxicity to *E. coli*. *Environ. Sci. Technol.* **2013**, *47* (11), 5738–5745.
- (43) Diegoli, S.; Manciuola, A. L.; Begum, S.; Jones, I. P.; Lead, J. R.; Preece, J. A. Interaction between manufactured gold nanoparticles and naturally occurring organic macromolecules. *Sci. Total Environ.* **2008**, *402* (1), 51–61.
- (44) Fabrega, J.; Luoma, S. N.; Tyler, C. R.; Galloway, T. S.; Lead, J. R. Silver nanoparticles: Behaviour and effects in the aquatic environment. *Environ. Int.* **2011**, *37* (2), 517–531.
- (45) Fabrega, J.; Fawcett, S. R.; Renshaw, J. C.; Lead, J. R. Silver nanoparticle impact on bacterial growth: effect of pH, concentration, and organic matter. *Environ. Sci. Technol.* **2009**, *43* (19), 7285–7290.
- (46) Fabrega, J.; Renshaw, J. C.; Lead, J. R. Interactions of silver nanoparticles with *Pseudomonas putida* biofilms. *Environ. Sci. Technol.* **2009**, *43* (23), 9004–9009.
- (47) Akaighe, N.; MacCusprie, R. I.; Navarro, D. A.; Aga, D. S.; Banerjee, S.; Sohn, M.; Sharma, V. K. Humic Acid-induced silver nanoparticle formation under environmentally relevant conditions. *Environ. Sci. Technol.* **2011**, *45* (9), 3895–3901.
- (48) Poda, A.; Kennedy, A.; Cuddy, M.; Bednar, A. Investigations of UV photolysis of PVP-capped silver nanoparticles in the presence and absence of dissolved organic carbon. *J. Nanopart. Res.* **2013**, *15* (5), 1673.
- (49) Fortner, J. D.; Lyon, D. Y.; Sayes, C. M.; Boyd, A. M.; Falkner, J. C.; Hotze, E. M.; Alemany, L. B.; Tao, Y. J.; Guo, W.; Ausman, K. D.; Colvin, V. L.; Hughes, J. B. C60 in Water: Nanocrystal formation and microbial response. *Environ. Sci. Technol.* **2005**, *39* (11), 4307–4316.
- (50) Cumberland, S. A.; Lead, J. R. Particle size distributions of silver nanoparticles at environmentally relevant conditions. *J. Chromatogr. A* **2009**, *1216* (52), 9099–105.
- (51) Petersen, E. J.; Pinto, R. A.; Mai, D. J.; Landrum, P. F.; Weber, W. J., Jr. Influence of polyethyleneimine graftings of multi-walled carbon nanotubes on their accumulation and elimination by and toxicity to *Daphnia magna*. *Environ. Sci. Technol.* **2011**, *45* (3), 1133–8.
- (52) Tervonen, K.; Waissi, G.; Petersen, E. J.; Akkanen, J.; Kukkonen, J. V. Analysis of fullerene-C60 and kinetic measurements for its accumulation and depuration in *Daphnia magna*. *Environ. Toxicol. Chem.* **2010**, *29* (5), 1072–1078.
- (53) Guo, X.; Dong, S.; Petersen, E. J.; Gao, S.; Huang, Q.; Mao, L. Biological uptake and depuration of radio-labeled graphene by *Daphnia magna*. *Environ. Sci. Technol.* **2013**, *47* (21), 12524–31.
- (54) Pakarinen, K.; Petersen, E. J.; Alvila, L.; Waissi-Leinonen, G. C.; Akkanen, J.; Leppanen, M. T.; Kukkonen, J. V. A screening study on the fate of fullerenes (nC60) and their toxic implications in natural freshwaters. *Environ. Toxicol. Chem.* **2013**, *32* (6), 1224–32.
- (55) Domingos, R. F.; Baalousha, M. A.; Ju-Nam, Y.; Reid, M. M.; Tufenkji, N.; Lead, J. R.; Leppard, G. G.; Wilkinson, K. J. Characterizing Manufactured Nanoparticles in the Environment: Multimethod Determination of Particle Sizes. *Environ. Sci. Technol.* **2009**, *43* (19), 7277–7284.
- (56) Baalousha, M.; Ju-Nam, Y.; Cole, P. A.; Gaiser, B.; Fernandes, T. F.; Hriljac, J. A.; Jepson, M. A.; Stone, V.; Tyler, C. R.; Lead, J. R. Characterization of cerium oxide nanoparticles—Part 1: Size measurements. *Environ. Toxicol. Chem.* **2012**, *31* (5), 983–993.
- (57) Baalousha, M.; Ju-Nam, Y.; Cole, P. A.; Hriljac, J. A.; Jones, I. P.; Tyler, C. R.; Stone, V.; Fernandes, T. F.; Jepson, M. A.; Lead, J. R. Characterization of cerium oxide nanoparticles—Part 2: Nonsize measurements. *Environ. Toxicol. Chem.* **2012**, *31* (5), 994–1003.
- (58) Römer, I.; Gavin, A. J.; White, T. A.; Merrifield, R. C.; Chipman, J. K.; Viant, M. R.; Lead, J. R. The critical importance of defined media conditions in *Daphnia magna* nanotoxicity studies. *Toxicol. Lett.* **2013**, *223* (1), 103–108.
- (59) Baalousha, M.; Prasad, A.; Lead, J. R. Quantitative measurement of the nanoparticle size and number concentration from liquid suspensions by atomic force microscopy. *Environ. Sci.: Processes Impacts* **2014**, *16* (6), 1338–1347.
- (60) Roberts, A. P.; Mount, A. S.; Seda, B.; Souther, J.; Qiao, R.; Lin, S. J.; Ke, P. C.; Rao, A. M.; Klaine, S. J. In vivo biomodification of lipid-coated carbon nanotubes by *Daphnia magna*. *Environ. Sci. Technol.* **2007**, *41* (8), 3025–3029.
- (61) Kennedy, A. J.; Hull, M. S.; Steevens, J. A.; Dontsova, K. M.; Chappell, M. A.; Gunter, J. C.; Weiss, C. A. Factors influencing the partitioning and toxicity of nanotubes in the aquatic environment. *Environ. Toxicol. Chem.* **2008**, *27* (9), 1932–1941.
- (62) Kim, K. T.; Edgington, A. J.; Klaine, S. J.; Cho, J. W.; Kim, S. D. Influence of multiwalled carbon nanotubes dispersed in natural organic matter on speciation and bioavailability of copper. *Environ. Sci. Technol.* **2009**, *43* (23), 8979–84.
- (63) Petersen, E. J.; Henry, T. B. Methodological considerations for testing the ecotoxicity of carbon nanotubes and fullerenes: review. *Environ. Toxicol. Chem.* **2012**, *31* (1), 60–72.
- (64) Schierz, A.; Parks, A. N.; Washburn, K. M.; Chandler, G. T.; Ferguson, P. L. Characterization and Quantitative Analysis of single-walled carbon nanotubes in the aquatic environment using near-infrared fluorescence spectroscopy. *Environ. Sci. Technol.* **2012**, *46* (22), 12262–12271.
- (65) Doudrick, K.; Corson, N.; Oberdörster, G.; Eder, A. C.; Herckes, P.; Halden, R. U.; Westerhoff, P. Extraction and quantification of carbon nanotubes in biological matrices with application to rat lung tissue. *ACS Nano* **2013**, *7* (10), 8849–8856.
- (66) Doudrick, K.; Herckes, P.; Westerhoff, P. Detection of carbon nanotubes in environmental matrices using programmed thermal analysis. *Environ. Sci. Technol.* **2012**, *46* (22), 12246–12253.
- (67) Hyung, H.; Fortner, J. D.; Hughes, J. B.; Kim, J.-H. Natural organic matter stabilizes carbon nanotubes in the aqueous phase. *Environ. Sci. Technol.* **2007**, *41* (1), 179–184.
- (68) Atha, D. H.; Wang, H.; Petersen, E. J.; Cleveland, D.; Holbrook, R. D.; Jaruga, P.; Dizdaroğlu, M.; Xing, B.; Nelson, B. C. Copper oxide nanoparticle mediated DNA damage in terrestrial plant models. *Environ. Sci. Technol.* **2012**, *46* (3), 1819–1827.
- (69) Liu, J.; Sonshine, D. A.; Shervani, S.; Hurt, R. H. Controlled release of biologically active silver from nanosilver surfaces. *ACS Nano* **2010**, *4* (11), 6903–6913.
- (70) Pornwilard, M.-M.; Siripinyanond, A. Field-flow fractionation with inductively coupled plasma mass spectrometry: past, present, and future. *J. Anal. At. Spectrom.* **2014**, *29* (10), 1739–1752.
- (71) Liu, J.; Murphy, K. E.; MacCusprie, R. I.; Winchester, M. R. Capabilities of single particle inductively coupled plasma mass spectrometry for the size measurement of nanoparticles: a case study on gold nanoparticles. *Anal. Chem.* **2014**, *86* (7), 3405–3414.
- (72) Cornelis, G.; Hasselov, M. A signal deconvolution method to discriminate smaller nanoparticles in single particle ICP-MS. *J. Anal. At. Spectrom.* **2014**, *29* (1), 134–144.
- (73) Mitrano, D. M.; Barber, A.; Bednar, A.; Westerhoff, P.; Higgins, C. P.; Ranville, J. F. Silver nanoparticle characterization using single particle ICP-MS (SP-ICP-MS) and asymmetrical flow field flow fractionation ICP-MS (AF4-ICP-MS). *J. Anal. At. Spectrom.* **2012**, *27* (7), 1131–1142.
- (74) Mitrano, D. M.; Leshner, E. K.; Bednar, A.; Monserud, J.; Higgins, C. P.; Ranville, J. F. Detecting nanoparticulate silver using single-particle inductively coupled plasma–mass spectrometry. *Environ. Toxicol. Chem.* **2012**, *31* (1), 115–121.

- (75) Montano, M. D.; Badiei, H. R.; Bazargan, S.; Ranville, J. F. Improvements in the detection and characterization of engineered nanoparticles using spICP-MS with microsecond dwell times. *Environ. Sci.: Nano* **2014**, *1* (4), 338–346.
- (76) Mader, B. T.; Ellefson, M. E.; Wolf, S. T. Measurements of nanomaterials in environmentally-relevant water matrices using liquid nebulization/differential mobility (LN/DMA) analysis. *Environ. Toxicol. Chem.* **2015**, *34* (4), 833–842.
- (77) Zhang, L.; Petersen, E. J.; Habteselassie, M. Y.; Mao, L.; Huang, Q. Degradation of multiwall carbon nanotubes by bacteria. *Environ. Pollut.* **2013**, *181*, 335–339.
- (78) Zhang, Y.; Chen, D.; Smith, M. A.; Zhang, B.; Pan, X. Selection of reliable reference genes in *Caenorhabditis elegans* for analysis of nanotoxicity. *PLoS One* **2012**, *7* (3), e31849.
- (79) Mattison, N. T.; O'Carroll, D. M.; Kerry Rowe, R.; Petersen, E. J. Impact of porous media grain size on the transport of multi-walled carbon nanotubes. *Environ. Sci. Technol.* **2011**, *45* (22), 9765–9775.
- (80) Petosa, A. R.; Jaisi, D. P.; Quevedo, I. R.; Elimelech, M.; Tufenkji, N. Aggregation and deposition of engineered nanomaterials in aquatic environments: role of physicochemical interactions. *Environ. Sci. Technol.* **2010**, *44* (17), 6532–6549.
- (81) Sayre, P.; Prothero, S.; Alwood, J. Nanomaterial risk assessment and management experiences related to worker health under the Toxic Substances Control Act. *J. Occup. Environ. Med.* **2011**, *53*, S98–S102.
- (82) Lynch, I.; Weiss, C.; Valsami-Jones, E. A strategy for grouping of nanomaterials based on key physico-chemical descriptors as a basis for safer-by-design NMs. *Nano Today* **2014**, *9* (3), 266–270.
- (83) Meesters, J. A. J.; Koelmans, A. A.; Quik, J. T. K.; Hendriks, A. J.; van de Meent, D. Multimedia modeling of engineered nanoparticles with SimpleBox4nano: model definition and evaluation. *Environ. Sci. Technol.* **2014**, *48* (10), 5726–5736.
- (84) Wiench, K.; Wohlleben, W.; Hisgen, V.; Radke, K.; Salinas, E.; Zok, S.; Landsiedel, R. Acute and chronic effects of nano- and non-nano-scale TiO₂ and ZnO particles on mobility and reproduction of the freshwater invertebrate *Daphnia magna*. *Chemosphere* **2009**, *76* (10), 1356–1365.
- (85) OECD Test No. 218: *Sediment-Water Chironomid Toxicity Using Spiked Sediment*; Organisation for Economic Co-operation and Development: Paris, 2004.
- (86) OECD Test No. 219: *Sediment-Water Chironomid Toxicity Using Spiked Water*; Organisation for Economic Co-operation and Development: Paris, 2004.
- (87) Römer, I.; White, T. A.; Baalousha, M.; Chipman, K.; Viant, M. R.; Lead, J. R. Aggregation and dispersion of silver nanoparticles in exposure media for aquatic toxicity tests. *J. Chromatogr. A* **2011**, *1218* (27), 4226–4233.
- (88) Rand, G. M. *Fundamentals of Aquatic Toxicology: Effects, Environmental Fate and Risk Assessment*, 2nd ed.; Taylor and Francis: Philadelphia, 1995.
- (89) Elendt, B.-P.; Bias, W.-R. Trace nutrient deficiency in *Daphnia magna* cultured in standard medium for toxicity testing. Effects of the optimization of culture conditions on life history parameters of *D. magna*. *Water Res.* **1990**, *24* (9), 1157–1167.
- (90) OECD Test No. 201: *Freshwater Alga and Cyanobacteria, Growth Inhibition Test*; Organisation for Economic Co-operation and Development: Paris, 2011.
- (91) Priester, J. H.; Singhal, A.; Wu, B.; Stucky, G. D.; Holden, P. A. Integrated approach to evaluating the toxicity of novel cysteine-capped silver nanoparticles to *Escherichia coli* and *Pseudomonas aeruginosa*. *Analyst* **2014**, *139* (5), 954–63.
- (92) Pokhrel, L. R.; Dubey, B.; Scheuerman, P. R. Impacts of select organic ligands on the colloidal stability, dissolution dynamics, and toxicity of silver nanoparticles. *Environ. Sci. Technol.* **2013**, *47* (22), 12877–12885.
- (93) Baumann, J.; Sakka, Y.; Bertrand, C.; Koser, J.; Filser, J. Adaptation of the *Daphnia sp.* acute toxicity test: miniaturization and prolongation for the testing of nanomaterials. *Environ. Sci. Pollut. Res.* **2014**, *21* (3), 2201–13.
- (94) Boyle, D.; Boran, H.; Atfield, A.; Henry, T. B. Use of an exposure chamber to maintain aqueous-phase nanoparticle dispersions for improved toxicity testing in fish. *Environ. Toxicol. Chem.* **2015**, *34* (3), 583–588.
- (95) Sekine, R.; Khurana, K.; Vasilev, K.; Lombi, E.; Donner, E. Quantifying the adsorption of ionic silver and functionalized nanoparticles during ecotoxicology testing: test container effects and recommendations. *Nanotoxicology* **2015**, *0*, 1–8.
- (96) OECD Test No. 211: *Daphnia magna Reproduction Test*; Organisation for Economic Co-operation and Development: Paris, 2012.
- (97) OCED Test No. 202: *Daphnia sp. Acute Immobilisation Test*; Organisation for Economic Co-operation and Development: Paris, 2004.
- (98) Xia, T.; Hamilton, R. F.; Bonner, J. C.; Crandall, E. D.; Elder, A.; Fazlollahi, F.; Girtsman, T. A.; Kim, K.; Mitra, S.; Ntim, S. A.; Orr, G.; Tagmount, M.; Taylor, A. J.; Telesca, D.; Tolic, A.; Vulpe, C. D.; Walker, A. J.; Wang, X.; Witzmann, F. A.; Wu, N.; Xie, Y.; Zink, J. I.; Nel, A.; Holian, A. Interlaboratory evaluation of in vitro cytotoxicity and inflammatory responses to engineered nanomaterials: the NIEHS Nano GO Consortium. *Environ. Health Persp.* **2013**, *121* (6), 683–90.
- (99) Linsinger, T. P.; Peters, R.; Weigel, S. International interlaboratory study for sizing and quantification of Ag nanoparticles in food simulants by single-particle ICPMS. *Anal. Bioanal. Chem.* **2014**, *406* (16), 3835–43.
- (100) Petersen, E. J. In Response: Measurement science challenges that complicate the assessment of the potential ecotoxicological risks of carbon nanomaterials—A governmental perspective. *Environ. Toxicol. Chem.* **2015**, *34* (5), 955–957.
- (101) Henry, T. B.; Menn, F. M.; Fleming, J. T.; Wilgus, J.; Compton, R. N.; Sayler, G. S. Attributing effects of aqueous C60 nano-aggregates to tetrahydrofuran decomposition products in larval zebrafish by assessment of gene expression. *Environ. Health Persp.* **2007**, *115* (7), 1059–65.
- (102) Henry, T. B.; Petersen, E. J.; Compton, R. N. Aqueous fullerene aggregates (nC60) generate minimal reactive oxygen species and are of low toxicity in fish: a revision of previous reports. *Curr. Opin. Biotechnol.* **2011**, *22* (4), 533–7.
- (103) Spohn, P.; Hirsch, C.; Hasler, F.; Bruinink, A.; Krug, H. F.; Wick, P. C60 fullerene: a powerful antioxidant or a damaging agent? The importance of an in-depth material characterization prior to toxicity assays. *Environ. Pollut.* **2009**, *157* (4), 1134–9.
- (104) Zhang, L.; Petersen, E. J.; Huang, Q. Phase Distribution of 14C-labeled multiwalled carbon nanotubes in aqueous systems containing model solids: peat. *Environ. Sci. Technol.* **2011**, *45* (4), 1356–1362.
- (105) Cupi, D.; Hartmann, N.; Baun, A. The influence of natural organic matter and aging on suspension stability in guideline toxicity testing of Ag, ZnO, and TiO₂ nanoparticles with *Daphnia magna*. *Environ. Toxicol. Chem.* **2015**, *34* (3), 497–506.
- (106) Versteeg, D. J.; Shorter, S. J. Effect of organic carbon on the uptake and toxicity of quaternary ammonium compounds to the fathead minnow, *Pimephales promelas*. *Environ. Toxicol. Chem.* **1992**, *11* (4), 571–580.
- (107) Thurman, E. M. *Organic Geochemistry of Natural Waters*; Developments in Biogeochemistry, Vol. 2; Martinus Nijhoff/Dr W. Junk Publishers: Dordrecht, The Netherlands, 1985.
- (108) Boethling, R. S.; Nabholz, J. V. *Environmental Assessment of Polymers under the U.S. Toxic Substances Control Act*; U.S. Environmental Protection Agency: Washington, DC, 1996.
- (109) Heller, D. A.; Barone, P. W.; Strano, M. S. Sonication-induced changes in chiral distribution: A complication in the use of single-walled carbon nanotube fluorescence for determining species distribution. *Carbon* **2005**, *43* (3), 651–653.
- (110) Kennedy, A. J.; Gunter, J. C.; Chappell, M. A.; Goss, J. D.; Hull, M. S.; Kirgan, R. A.; Steevens, J. A. Influence of nanotube preparation in aquatic bioassays. *Environ. Toxicol. Chem.* **2009**, *28* (9), 1930–1938.

- (111) Petersen, E. J.; Tu, X.; Dizdaroglu, M.; Zheng, M.; Nelson, B. C. Protective roles of single-wall carbon nanotubes in ultrasonication-induced DNA base damage. *Small* **2013**, *9* (2), 205–208.
- (112) Wang, R.; Hughes, T.; Beck, S.; Vakil, S.; Li, S.; Pantano, P.; Draper, R. K. Generation of toxic degradation products by sonication of Pluronic(R) dispersants: implications for nanotoxicity testing. *Nanotoxicology* **2013**, *7* (7), 1272–81.
- (113) Betts, J. N.; Johnson, M. G.; Rygielwicz, P. T.; King, G. A.; Andersen, C. P. Potential for metal contamination by direct sonication of nanoparticle suspensions. *Environ. Toxicol. Chem.* **2013**, *32* (4), 889–893.
- (114) Carr, R.; Nipper, M.; Adams, W.; Berry, W.; Burton, G.; Ho, K.; MacDonald, D.; Scroggins, R.; Winger, P. *Summary of a SETAC Technical Workshop: Pore Water Toxicity Testing: Biological, Chemical, and Ecological Considerations with a Review of Methods and Applications, and Recommendations for Future Areas of Research*; SETAC: Pensacola, FL, 2001.
- (115) Ruffli, H.; Fisk, P. R.; Girling, A. E.; King, J. M. H.; Länge, R.; Lejeune, X.; Stelter, N.; Stevens, C.; Suteau, P.; Tapp, J.; Thus, J.; Versteeg, D. J.; Niessen, H. J. Aquatic toxicity testing of sparingly soluble, volatile, and unstable substances and interpretation and use of data. *Ecotoxicol. Environ. Saf.* **1998**, *39* (2), 72–77.
- (116) Girling, A. E.; Whale, G. F.; Adema, D. M. M. A guideline supplement for determining the aquatic toxicity of poorly water-soluble complex mixtures using water-accommodated fractions. *Chemosphere* **1994**, *29* (12), 2645–2649.
- (117) Park, J. W.; Oh, J. H.; Kim, W. K.; Lee, S. K. Toxicity of citrate-coated silver nanoparticles differs according to method of suspension preparation. *Bull. Environ. Contam. Toxicol.* **2014**, *93* (1), 53–9.
- (118) Ong, K. J.; MacCormack, T. J.; Clark, R. J.; Ede, J. D.; Ortega, V. A.; Felix, L. C.; Dang, M. K. M.; Ma, G.; Fenniri, H.; Veinot, J. G. C.; Goss, G. G. Widespread nanoparticle-assay interference: implications for nanotoxicity testing. *PLoS One* **2014**, *9* (3), e90650.
- (119) Hartmann, N. B.; Engelbrekt, C.; Zhang, J.; Ulstrup, J.; Kusk, K. O.; Baun, A. The challenges of testing metal and metal oxide nanoparticles in algal bioassays: titanium dioxide and gold nanoparticles as case studies. *Nanotoxicology* **2013**, *7* (6), 1082–94.
- (120) Petersen, E. J.; Reipa, V.; Watson, S. S.; Stanley, D. L.; Rabb, S. A.; Nelson, B. C. DNA damaging potential of photoactivated P25 titanium dioxide nanoparticles. *Chem. Res. Toxicol.* **2014**, *27* (10), 1877–1884.
- (121) OECD Test No. 203: *Fish, Acute Toxicity Test*; Organisation for Economic Co-operation and Development: Paris, 1992.
- (122) OECD Test No. 221: *Lemna sp. Growth Inhibition Test*; Organisation for Economic Co-operation and Development: Paris, 2006.
- (123) *Ecological Effects Test Guidelines - OPPTS 850.1000 Special Considerations for Conducting Aquatic Laboratory Studies*; U.S. Environmental Protection Agency: Washington, DC, 1996.
- (124) Oberdörster, G. Significance of particle parameters in the evaluation of exposure-dose-response relationships of inhaled particles. *Part. Sci. Technol.* **1996**, *14* (2), 135–151.
- (125) Donaldson, K.; Li, X. Y.; MacNee, W. Ultrafine (nanometre) particle mediated lung injury. *J. Aerosol Sci.* **1998**, *29* (5–6), 553–560.
- (126) Sondi, I.; Salopek-Sondi, B. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *J. Colloid Interface Sci.* **2004**, *275* (1), 177–182.
- (127) Morones, J. R.; Elechiguerra, J. L.; Camacho, A.; Holt, K.; Kouri, J. B.; Ramirez, J. T.; Yacaman, M. J. The bactericidal effect of silver nanoparticles. *Nanotechnology* **2005**, *16* (10), 2346.
- (128) Oberdörster, G.; Oberdörster, E.; Oberdörster, J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ. Health Persp.* **2005**, *113* (7), 823–839.
- (129) Choi, O.; Hu, Z. Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. *Environ. Sci. Technol.* **2008**, *42* (12), 4583–4588.
- (130) Griffitt, R. J.; Hyndman, K.; Denslow, N. D.; Barber, D. S. Comparison of molecular and histological changes in zebrafish gills exposed to metallic nanoparticles. *Toxicol. Sci.* **2009**, *107* (2), 404–415.
- (131) Pal, S.; Tak, Y. K.; Song, J. M. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium. *Appl. Environ. Microb.* **2007**, *73* (6), 1712–1720.
- (132) Donaldson, K.; Poland, C. A. Nanotoxicity: challenging the myth of nano-specific toxicity. *Curr. Opin. Biotechnol.* **2013**, *24* (4), 724–734.
- (133) Landsiedel, R. Nanotechnology – safety first. *Chem. Ind.* **2014**, *78* (9), 28–28.
- (134) Cowart, D. A.; Guida, S. M.; Shah, S. I.; Marsh, A. G. Effects of Ag nanoparticles on survival and oxygen consumption of zebra fish embryos, *Danio rerio*. *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.* **2011**, *46* (10), 1122–1128.
- (135) Hoheisel, S. M.; Diamond, S.; Mount, D. Comparison of nanosilver and ionic silver toxicity in *Daphnia magna* and *Pimephales promelas*. *Environ. Toxicol. Chem.* **2012**, *31* (11), 2557–2563.
- (136) Kennedy, A. J.; Chappell, M. A.; Bednar, A. J.; Ryan, A. C.; Laird, J. G.; Stanley, J. K.; Steevens, J. A. Impact of organic carbon on the stability and toxicity of fresh and stored silver nanoparticles. *Environ. Sci. Technol.* **2012**, *46* (19), 10772–10780.
- (137) Kennedy, A. J.; Hull, M. S.; Bednar, A. J.; Goss, J. D.; Gunter, J. C.; Bouldin, J. L.; Vikesland, P. J.; Steevens, J. A. Fractionating nanosilver: importance for determining toxicity to aquatic test organisms. *Environ. Sci. Technol.* **2010**, *44* (24), 9571–9577.
- (138) Li, T.; Albee, B.; Alemayehu, M.; Diaz, R.; Ingham, L.; Kamal, S.; Rodriguez, M.; Bishnoi, S. W. Comparative toxicity study of Ag, Au, and Ag–Au bimetallic nanoparticles on *Daphnia magna*. *Anal. Bioanal. Chem.* **2010**, *398* (2), 689–700.
- (139) Gao, J.; Youn, S.; Hovsepian, A.; Llana, V. L.; Wang, Y.; Bitton, G.; Bonzongo, J.-C. J. Dispersion and toxicity of selected manufactured nanomaterials in natural river water samples: effects of water chemical composition. *Environ. Sci. Technol.* **2009**, *43* (9), 3322–3328.
- (140) Hull, M.; Kennedy, A. J.; Detzel, C.; Vikesland, P.; Chappell, M. Moving beyond mass: the unmet need to consider dose metrics in environmental nanotoxicology studies. *Environ. Sci. Technol.* **2012**, *46* (20), 10881–10882.
- (141) Kennedy, A.; Diamond, D.; Stanley, J. K.; Coleman, J.; Steevens, J. A.; Laird, J. G. Nanomaterials ecotoxicology: a case study with nanosilver. In *Nanotechnology Risk Management*; Hull, M., Bowman, D., Eds.; Springer: London, 2014.
- (142) Van Hoecke, K.; De Schampelaere, K. A.; Van der Meeren, P.; Lucas, S.; Janssen, C. R. Ecotoxicity of silica nanoparticles to the green alga *Pseudokirchneriella subcapitata*: importance of surface area. *Environ. Toxicol. Chem.* **2008**, *27* (9), 1948–1957.
- (143) Van Hoecke, K.; Quik, J. T.; Mankiewicz-Boczek, J.; De Schampelaere, K. A.; Elsaesser, A.; Van der Meeren, P.; Barnes, C.; McKerr, G.; Howard, C. V.; Van De Meent, D.; et al. Fate and effects of CeO₂ nanoparticles in aquatic ecotoxicity tests. *Environ. Sci. Technol.* **2009**, *43* (12), 4537–4546.
- (144) Yang, X.; Gondikas, A. P.; Marinakos, S. M.; Auffan, M.; Liu, J.; Hsu-Kim, H.; Meyer, J. N. Mechanism of silver nanoparticle toxicity is dependent on dissolved silver and surface coating in *Caenorhabditis elegans*. *Environ. Sci. Technol.* **2012**, *46* (2), 1119–1127.
- (145) Jo, H. J.; Choi, J. W.; Lee, S. H.; Hong, S. W. Acute toxicity of Ag and CuO nanoparticle suspensions against *Daphnia magna*: the importance of their dissolved fraction varying with preparation methods. *J. Hazard. Mater.* **2012**, *227–228*, 301–308.
- (146) Newton, K. M.; Puppala, H. L.; Kitchens, C. L.; Colvin, V. L.; Klaire, S. J. Silver nanoparticle toxicity to *Daphnia magna* is a function of dissolved silver concentration. *Environ. Toxicol. Chem.* **2013**, *32* (10), 2356–64.
- (147) Auffan, M.; Rose, J.; Bottero, J.-Y.; Lowry, G. V.; Jolivet, J.-P.; Wiesner, M. R. Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. *Nat. Nanotechnol.* **2009**, *4* (10), 634–641.

- (148) Phalen, R. F.; Mendez, L. B.; Oldham, M. J. New developments in aerosol dosimetry. *Inhalation Toxicol.* **2010**, *22* (S2), 6–14.
- (149) Grass, R. N.; Limbach, L. K.; Athanassiou, E. K.; Stark, W. J. Exposure of aerosols and nanoparticle dispersions to *in vitro* cell cultures: a review on the dose relevance of size, mass, surface and concentration. *J. Aerosol Sci.* **2010**, *41* (12), 1123–1142.
- (150) Wittmaack, K. In search of the most relevant parameter for quantifying lung inflammatory response to nanoparticle exposure: particle number, surface area, or what? *Environ. Health Persp.* **2007**, *115* (2), 187–194.
- (151) Brown, D. M.; Wilson, M. R.; MacNee, W.; Stone, V.; Donaldson, K. Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. *Toxicol. Appl. Pharmacol.* **2001**, *175* (3), 191–199.
- (152) Kennedy, A. J.; Melby, N. L.; Moser, R. D.; Bednar, A. J.; Son, S. F.; Lounds, C. D.; Laird, J. G.; Nellums, R. R.; Johnson, D. R.; Steevens, J. A. Fate and toxicity of CuO nanospheres and nanorods used in Al/CuO nanothermites before and after combustion. *Environ. Sci. Technol.* **2013**, *47* (19), 11258–11267.
- (153) Pompa, P.; Vecchio, G.; Galeone, A.; Brunetti, V.; Maiorano, G.; Sabella, S.; Cingolani, R. Physical assessment of toxicology at nanoscale: nano dose-metrics and toxicity factor. *Nanoscale* **2011**, *3* (7), 2889–2897.
- (154) OECD Test No. 225: *Sediment-Water Lumbriculus Toxicity Test Using Spiked Sediment*; Organisation for Economic Co-operation and Development: Paris, 2007.
- (155) Kukkonen, J. V.; Landrum, P. F. Effect of particle-xenobiotic contact time on bioavailability of sediment-associated benzo (a) pyrene to benthic amphipod. *Aquat. Toxicol.* **1998**, *42* (3), 229–242.
- (156) Petersen, E. J.; Huang, Q.; Weber, W. Ecological uptake and depuration of carbon nanotubes by *Lumbriculus variegatus*. *Environ. Health Persp.* **2008**, *116* (4), 496–500.
- (157) Pakarinen, K.; Petersen, E.; Leppänen, M.; Akkanen, J.; Kukkonen, J. Adverse effects of fullerenes (nC60) spiked to sediments on *Lumbriculus variegatus* (*Oligochaeta*). *Environ. Pollut.* **2011**, *159* (12), 3750–3756.
- (158) Waissi-Leinonen, G. C.; Petersen, E. J.; Pakarinen, K.; Akkanen, J.; Leppänen, M. T.; Kukkonen, J. V. Toxicity of fullerene (C60) to sediment-dwelling invertebrate *Chironomus riparius* larvae. *Environ. Toxicol. Chem.* **2012**, *31* (9), 2108–2116.
- (159) Stanley, J. K.; Coleman, J. G.; Weiss, C. A.; Steevens, J. A. Sediment toxicity and bioaccumulation of nano and micron-sized aluminum oxide. *Environ. Toxicol. Chem.* **2010**, *29* (2), 422–429.
- (160) ASTM E139-94: Standard guide for collection, storage, characterization, and manipulation of sediments for toxicological testing. In *ASTM Standards on Environmental Sampling*, Vol. 11.05; American Society for Testing and Materials: Conshohocken, PA, 2000.
- (161) *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates*, 2nd ed.; U.S. Environmental Protection Agency: Duluth, MN, 2000.
- (162) *Methods for Collection, Storage and Manipulation of Sediments for Chemical and Toxicological Analyses: Technical Manual*; U.S. Environmental Protection Agency: Washington, DC, 2001.
- (163) OECD Test No. 233: *Sediment-Water Chironomid Life-Cycle Toxicity Test Using Spiked Water or Spiked Sediment*; Organisation for Economic Co-operation and Development: Paris, 2010.
- (164) Simpson, S. L.; Angel, B. M.; Jolley, D. F. Metal equilibration in laboratory-contaminated (spiked) sediments used for the development of whole-sediment toxicity tests. *Chemosphere* **2004**, *54* (5), 597–609.
- (165) *Guidance Document on Transformation/Dissolution of Metals and Metal Compounds in Aqueous Media*; Organisation for Economic Co-operation and Development: Paris, 2002.
- (166) Harmon, A. R.; Kennedy, A. J.; Poda, A. R.; Bednar, A. J.; Chappell, M. A.; Steevens, J. A. Determination of nanosilver dissolution kinetics and toxicity in an environmentally relevant aqueous medium. *Environ. Toxicol. Chem.* **2014**, *33* (8), 1783–1791.